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Research Note

Scanning and Transmission Electron Microscopic Observations on Metacercariae of *Echinostoma trivolvis* and *Echinostoma caproni* During In Vitro Excystation

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ABSTRACT: Electron microscopy was used to study the metacercariae of *Echinostoma trivolvis* and *Echinostoma caproni* during in vitro excystation. Untreated cysts of both species possessed a particulate outer layer, an amorphous middle layer, and a lamellated inner layer. Larvae of cysts treated in an alkaline bile salts–trypsin medium first emerged into a space between the outer and inner layers. Breaching of the inner cyst layer by the larva involved fraying at 1 point so that the lamellated layer spread to become more diffuse. Globules found in close proximity to this region may be secretions implicated in the disruption of the lamellated layer. Prior to final emergence, the outer layer was breached. The only differences in the 2 species were that *E. trivolvis* had a thicker middle layer and the surface of its inner layer was rougher than that of *E. caproni.*

KEY WORDS: Trematoda, Digenea, *Echinostoma trivolvis, Echinostoma caproni,* in vitro excystation, scanning electron microscopy, transmission electron microscopy.

According to Kanev (1985), for 37-collar-spined echinostomes, *Echinostoma caproni* is the correct name for a species described by Jeyarasasingam et al. (1972) as *Echinostoma liei,* and *Echinostoma trivolvis* is the correct name for a North American form worked with by Beaver (1937) as *Echinostoma revolutum.* Our study uses the Egyptian and North American species and refers to them as *E. caproni* and *E. trivolvis,* respectively. A recent review by Christensen et al. (1988) has discussed fundamental differences in the biology of these 2 related species. However, there is sparse ultrastructural information on larval or adult stages of these 2 echinostomes.

Fried and Emili (1988) examined chemical excystation of the 2 related 37-collar-spined echinostomes by light microscopy. Only subtle differences between the 2 species were observed in the morphology of metacercariae, i.e., width of the outer cyst wall, diameter of the excretory concretions, and length of the excysted larvae. The present study uses scanning and transmission electron microscopy to show similarities and differences in the ultrastructure of the metacercariae of *E. trivolvis* and *E. caproni* during in vitro excystation.

Metacercarial cysts of both echinostome species were maintained in laboratory-infected snails, *Biomphalaria glabrata,* as described by Jeyara-

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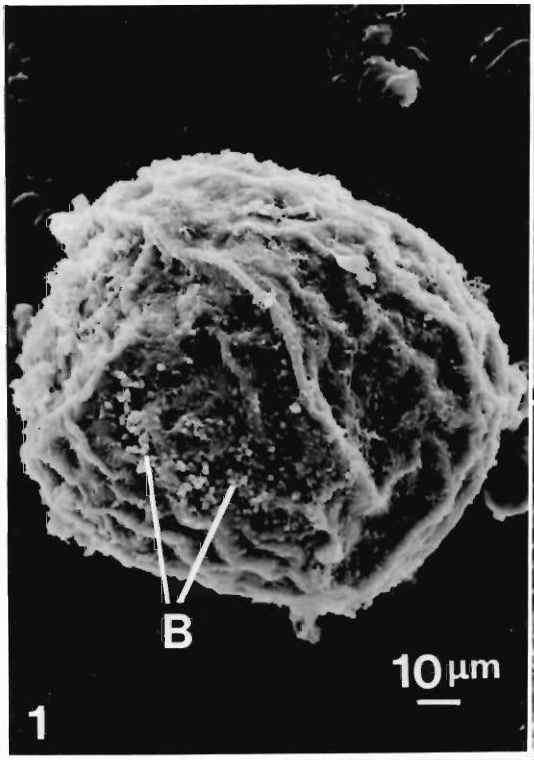
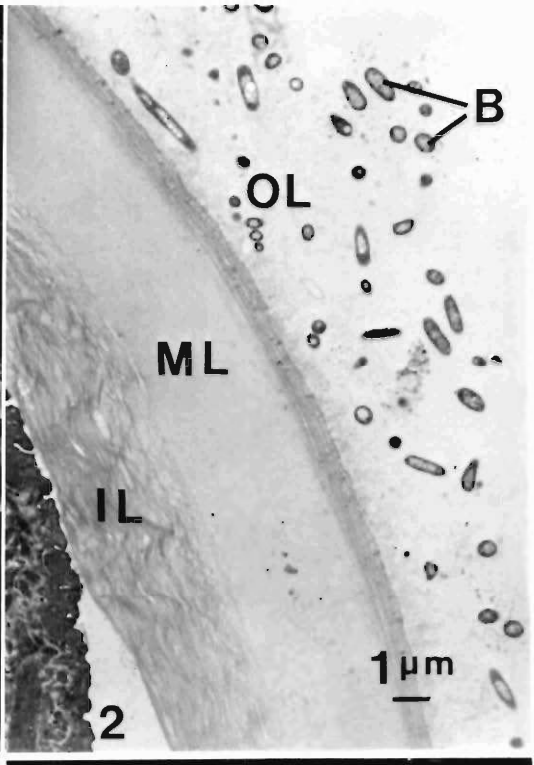
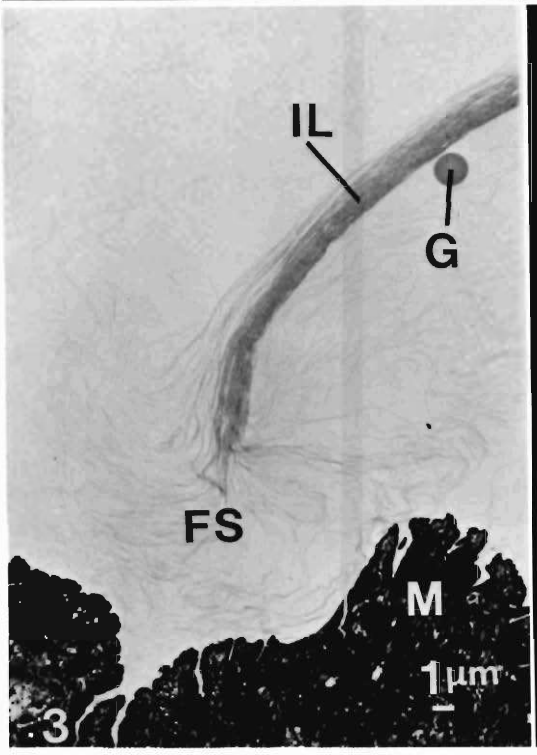
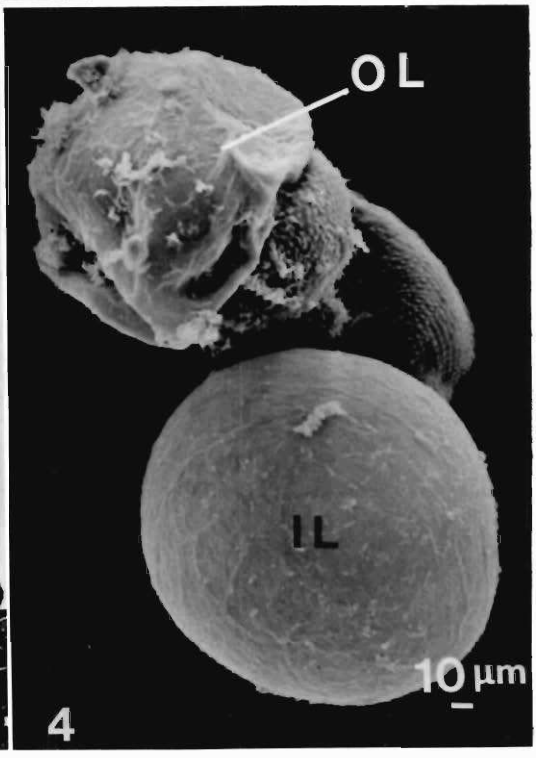
Figures 1–4. Electron micrographs of *Echinostoma trivolvis*. 1. Scanning electron micrograph of a whole cyst. Bacteria can be seen adhering to the outer surface. 2. Transmission electron micrograph of the untreated cyst wall showing the inner lamellated layer, the amorphous middle layer, and the outer layer, which has collagen-like material containing bacteria on the outside and is lamellated on the inner aspect. 3. The frayed structure of a breached inner layer. Part of a metacercaria can be seen passing through the breach. Note the presence of an electron-dense globule in the area. 4. This specimen is emerging from its inner cyst layer. The outer layer is still encapsulating the anterior end of the fluke. Abbreviations: B, bacteria; FR, frayed region; FS, frayed structure; G, globule; IL, inner layer; M, metacercaria; ML, middle layer; OL, outer layer.

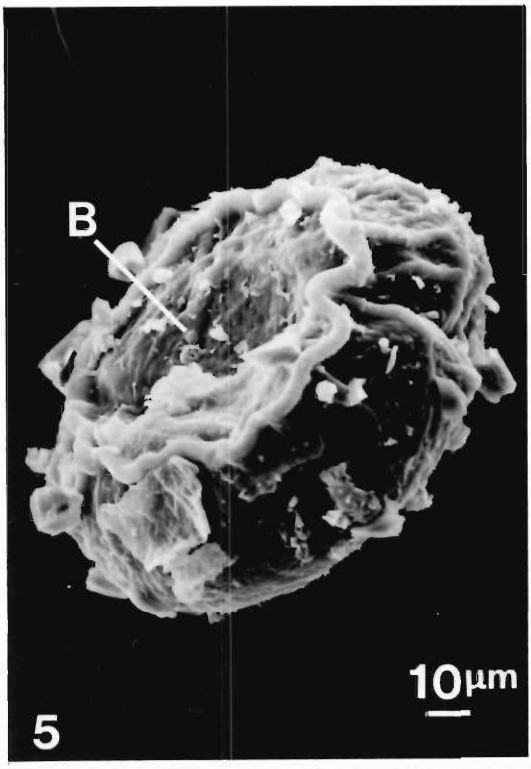
Figures 5–8. Electron micrographs of *Echinostoma caproni*. 5. A scanning electron micrograph of a whole cyst. A few bacteria are present on its surface. 6. The inner lamellated layer, middle amorphous layer, and outer layer, which is lamellated at its base and has collagen-like particles containing a bacterium on its outer aspect. 7. The frayed region of the inner layer of a breached cyst. A number of electron-dense globules are present close to the frayed region. 8. A metacercaria having emerged from the very smooth inner layer. The edge of the escape aperture (arrows) is thickened and frayed. A discarded outer layer remains nearby. Abbreviations: B, bacteria; FR, frayed region; FS, frayed structure; G, globule; IL, inner layer; M, metacercaria; ML, middle layer; OL, outer layer.

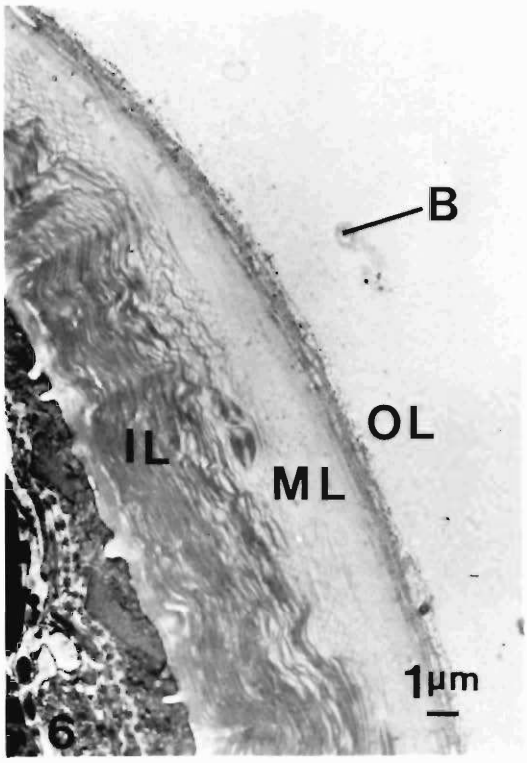


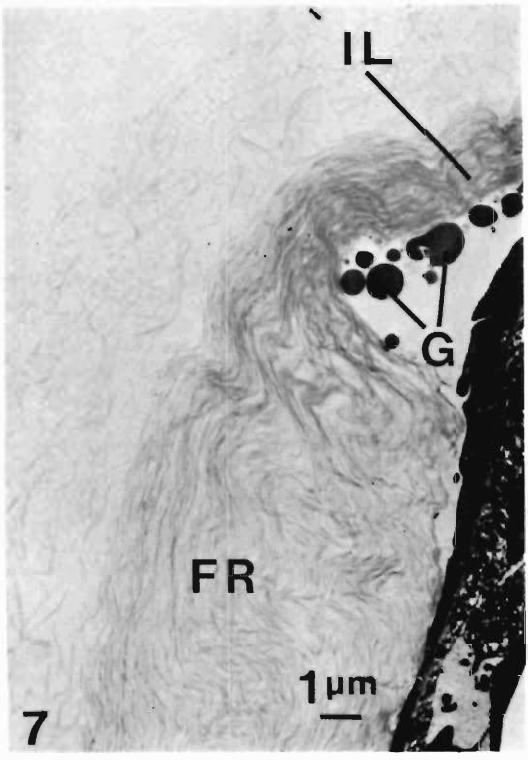


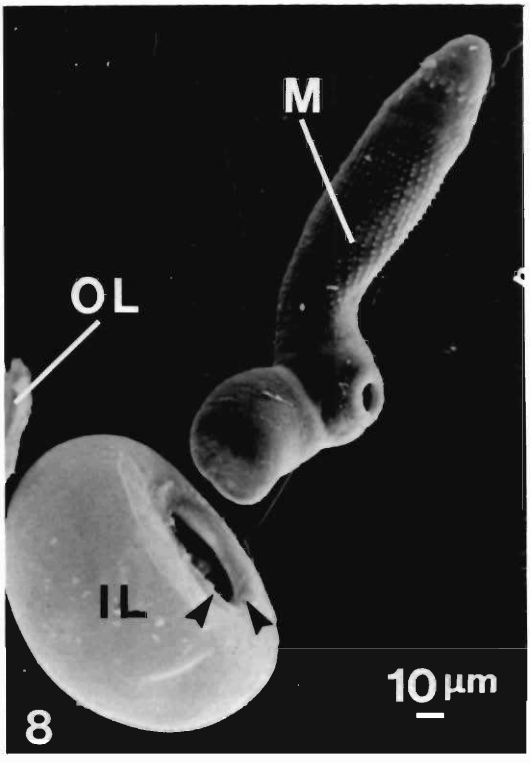












sasingam et al. (1972) and Anderson and Fried (1987) and were dissected from the saccular kidney 1–10 days postencystment. Both species of metacercariae were excysted following the method of Fried and Emili (1988) using an alkaline medium containing trypsin and bile salts at 41°C, and organisms at various stages of excystment were fixed along with untreated metacercarial cysts for examination by electron microscopy. Preparation of material followed procedures adopted by Irwin et al. (1984) and observations were made using JEOL 100S and JEOL JSM-840 electron microscopes.

Electron micrographs of *E. trivolvis* are shown in Figures 1–4 and of *E. caproni* in Figures 5–8. Scanning electron microscopy demonstrated that untreated cysts of *E. trivolvis* and *E. caproni* had a wrinkled or folded appearance; bacteria were observed on the cyst surfaces (Figs. 1, 5). Transmission electron microscopy of both species showed that untreated cysts had an inner lamellated layer merging into a middle layer of uniformly dense material that was thicker in *E. trivolvis* (Fig. 2) than in *E. caproni* (Fig. 6). The peripheral aspect of the outer layer had a particulate appearance that resembled collagen fibers and was somewhat lamellated toward its base where it merged with the amorphous middle layer. Sections through walls of metacercarial cysts of both species following exposure to the excystation medium demonstrated that much of the collagen-like material on the outside of the cyst had gone. The lamellated base of the outer layer and the inner lamellated layer were relatively unchanged, whereas the intervening amorphous layer (middle layer) was either diminished or not present. Breaching of the inner cyst layer by the larva involved a fraying and fragmenting at 1 point on the cyst wall so that the lamellated inner layer widened and became more diffuse (Figs. 3, 7). The organism passed through the frayed area, and in both species small electron-dense globules were often found in close proximity to the breached area (Figs. 3, 7). As the larva entered the cavity between the outer and inner cyst layers, fragments of the inner lamellated layer were carried into that space. The larvae at this stage were still constrained by the outer cyst layer. Excystation was completed by the disruption of the outer cyst layer. In some cases it was breached and the organism passed directly through the ruptured zone. In other cases the outer layer was separated from the inner layer, and the larvae could be observed becoming free as they passed through the now well-dispersed fragments of the inner lamellated layer. The outer layer could be retained for a short time as a cap over the anterior end of the escaping larva (Fig. 4), and eventually these outer layers were left scattered among the vacated and semivacated inner layers (Fig. 8). Those inner layers, which were now devoid of their outer covering, had markedly smooth surfaces although those of *E. caproni* were somewhat smoother than those of *E. trivolvis*. Scanning electron microscopy also demonstrated that vacated inner layers were somewhat flattened on the side from which the larvae escaped (Fig. 4). In each case the edge of the aperture was everted and had a ragged appearance consistent with the frayed and fragmented lamellar configuration demonstrated by transmission electron microscopy (Figs. 3, 7).

Breaching of the inner layer and the presence of electron-dense globules only at the ruptured site of the cysts in both species suggest that the organisms play an important role in excystment. The fraying and widening of the inner layer probably represented weakening that allowed the larva to breach this area. The globules may be glandular secretions involved in the disruption of the lamellated layer. The only other ultrastructural study on excystation of an echinostome was by Irwin et al. (1984) on *Himasthla leptosoma*. It showed that *H. leptosoma* metacercariae escape through regions of the inner cyst wall without lamellae. Unlike *E. trivolvis* and *E. caproni*, which break through the lamellated layer, no fraying or fragmenting of lamellae was observed in that species.

Although a previous study (Fried and Emili, 1988) demonstrated physiological differences in excystation and subtle morphologic differences in encysted and excysted metacercariae of *E. trivolvis* and *E. caproni*, the present ultrastructural study failed to demonstrate any differences in the excystation process. Indeed the only differences revealed by electron microscopy were the relative thickness of the middle cyst layer of *E. trivolvis* compared to *E. caproni* and the relative coarseness of the inner layer surface of *E. trivolvis* compared to that of *E. caproni*. Although these 2 echinostomes are distinct species, differences in larval morphology at the electron microscope level are not readily apparent in intact cysts or those undergoing excystation. It is apparent that EM studies alone on species of closely related

echinostome metacercariae may not be sufficient for precise identification solely on the basis of morphology.

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Helminths of the Arizona Little Striped Whiptail, *Cnemidophorus inornatus arizonae*, and the Desert Grassland Whiptail, *Cnemidophorus uniparens* (Sauria: Teiidae), from Southeastern Arizona

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ABSTRACT: Examination of the gastrointestinal tract of 78 *Cnemidophorus inornatus arizonae* Van Denburgh, 1896, revealed the presence of the nematodes, *Pharyngodon warneri* Harwood, 1932, and *Physaloptera* sp. Rudolphi, 1819, and a cestode, *Oochoristica bivitellobata* Loewen, 1940. Overall prevalence of infection was 33%. The highest prevalence and mean intensity was for *P. warneri,* 23% and 15.4, respectively. Examination of the gastrointestinal tract of 31 *Cnemidophorus uniparens* Wright and Lowe, 1965, revealed only the cestode *O. bivitellobata*; prevalence was 26% and mean intensity was 2.1. One juvenile acanthocephalan, *Acanthocephalus* sp. Koelreuther, 1771, was also found. Presence of *Physaloptera* sp., O. *bivitellobata,* and *Acanthocephalus* sp. are new host records.

KEY WORDS: Nematoda, Cestoda, Acanthocephala, prevalence, intensity, survey, Teiidae, *Cnemidophorus inornatus arizonae, Cnemidophorus uniparens.*

The Arizona little striped whiptail, *Cnemidophorus inornatus arizonae* Van Denburgh, 1896, occurs in arid and semiarid grasslands of western New Mexico and southeastern Arizona (Behler and King, 1979). The desert grassland whiptail, *Cnemidophorus uniparens* Wright and Lowe, 1965, occurs in desert scrub from central Arizona through southern New Mexico to El Paso, Texas, and south into Chihuahua, Mexico (Steb-